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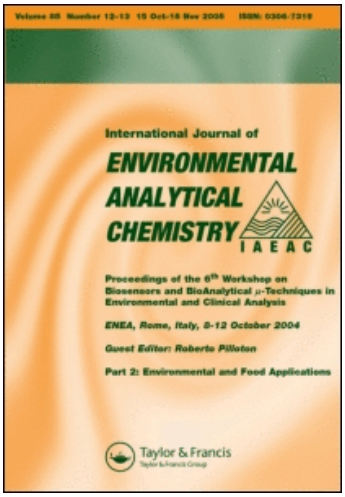

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The Use of Dechlorination in the Analysis of Polychlorinated Biphenyls and Related Classes of Compounds

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The use of dechlorination and subsequent analysis by means of column liquid chromatography (LC) as a quantitative, or qualitative, method of analysis for polychlorinated biphenyls (PCBs) and terphenyls (PCTs) and hydroxylated PCBs has been evaluated.

A relatively simple dechlorination procedure with $LiAlH_4$ as reagent has been developed and applied to various types of PCB-containing environmental samples, analysis being carried out by adsorption LC in the system silica gel/hexane with UV detection. The results are compared with those obtained by means of the gas chromatographic pattern-comparison and perchlorination methods. Preliminary data are presented on the use of dechlorination for the analysis of PCTs in environmental samples. Besides, the utilization of dechlorination as an aid for structure elucidation in metabolic studies of PCBs is reported.

KEY WORDS: (hydro)dechlorination, (hydroxylated) polychlorinated biphenyls, polychlorinated terphenyls, HPLC, perchlorination.

INTRODUCTION

In the past decade, increasing attention has been given to the identification and quantitative determination of polychlorinated biphenyls (PCBs) in environmental samples. Analysis is almost invariably done on a gas chromatograph (GC) equipped with an electron-capture detector (ECD). After a suitable, and often elaborate, clean-up procedure, analysis is performed by matching an arbitrary number of peaks in the chromatogram of the purified extract to those of the nearest commercially

available PCB formulation and measuring the height, or area, of the peaks. Perchlorination of all individual PCBs to the fully chlorinated decachlorobiphenyl (DCB) has repeatedly been propagated[1–8] as an alternative means of analysis, since it eliminates separation and interference problems, and simultaneously increases sensitivity and speeds up the actual quantitation procedure. It has, however, also been suggested that perchlorination may lead to gross errors due to the conversion of sample constituents other than PCBs into either DCB or products having gas chromatographic characteristics closely analogous to those of DCB. In another paper,[9] various examples of such errors are extensively discussed and the use of dechlorination of all PCBs to biphenyl as an alternative is shortly commented upon. In the present communication, a more systematic study on the scope and limitations of dechlorination of PCBs and related classes of compounds as an analytical tool is presented.

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EXPERIMENTAL

Chemicals

All commercial PCB and PCT mixtures from the Aroclor series—which were produced by Monsanto (St. Louis, Mo., U.S.A.)—as well as the commercial PCN mixtures—which were produced by Koppers (Pittsburgh, Pa., U.S.A.)—were obtained from Analabs (North Haven, Conn., U.S.A.). Borg-Warner Chemicals (Amsterdam, The Netherlands) and Péchiney-Ugine-Kuhlmann (Paris, France) supplied samples of Fire-Master BP-6 and decabromobiphenyl, respectively. All (chlorinated) hydroxybiphenyls were obtained from RFR Corp. (Hope, R.I., U.S.A.). Biphenyl was purchased from Aldrich Europe (Beers, Belgium), lithium aluminium hydride from Baker (Deventer, The Netherlands) and n-dodecane from Merck (Darmstadt, G.F.R.). ChromAR-grade hexane (Mallinckrodt, St. Louis, Mo., U.S.A.) was used as solvent in LC, while Nanograde hexane (Mallinckrodt) was found to be more suitable for GC work. Anhydrous diethyl ether (Baker) was distilled over $LiAlH_4$ and stored on molecular sieve.

Apparatus

The LC equipment consisted of an Orlita (Giessen, G.F.R.) Model FE 034 5RC reciprocating pump, a Valco (Houston, Texas, U.S.A.) six-port injection valve with a 100-μl loop, a stainless-steel column (25 cm $\times$ 3 mm I.D.) pre-packed with 5-μm LiChrosorb SI 60 silica gel (Merck), and a Pye-Unicam LC-3 variable-wavelength UV detector (Philips, Eindhoven, The Netherlands). Dry hexane was used as mobile phase. In order to

maintain a constant low water content of the chromatographic system, the solvent was dried on molecular sieve 5A; besides, a guard column filled with molecular sieve was inserted between the mobile-phase reservoir and the injection valve. Under such conditions, biphenyl displays a capacity ratio of 10–11. If this value starts to decrease significantly, the molecular sieve has to be replaced.

Analysis of the hydroxylated biphenyls was done with an LC system consisting of an Orlita Model AE 1044 pump, a Valco six-port injection valve with a 100-μl loop, a stainless-steel column (25 cm × 3 mm I.D.) prepacked with 7-μm LiChrosorb RP-8 (Merck) and a Pye-Unicam LC-3 UV detector. Methanol-water mixtures of varying composition were used as mobile phase. All LC experiments were carried out at ambient temperature.

A brief summary of conditions used in GC is as follows. A Packard–Becker (Delft, The Netherlands) Model 419 and a Pye GCV (Philips) equipped with ^{63}Ni ECDs were used as gas chromatographs. Analyses were performed on 2 m (pattern comparison) or 1 m (DCB after perchlorination) long glass columns packed with 4% OV-101 on Chromosorb W HP (80–100 mesh). Various temperature programs, typically ranging from 200 to 300°C, were used. All details of the GC procedure are reported elsewhere.[9]

As regards dechlorination, a Heraeus (Hanau, G.F.R.) Model T 5042 E oven (temperature limit, 250°C) was used for carrying out reactions according to Method I (see below). Reactions in screw-capped glass tubes (Method II) were done in an oil-bath, which was provided with an isolation jacket constructed from a number of 5-cm high plastic rings (diameter, ca. 25 cm). The glass tubes which were partly immersed in the oil bath, fit snugly in holes made in the cover ring and rest on their screw caps. This rather complicated mode of heating was necessary in order to prevent leaking problems and/or damage to the PTFE-lined screw caps—which cannot withstand too high a temperature—while still maintaining an efficient heating of the upper part of the glass tubes.

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Dechlorination

Method I. 1.0 ml of n-dodecane is added to a long and thick-walled glass reaction tube, the dimensions of which are given in Figure 1–1. Next, the extract of a PCB- or PCT-containing sample dissolved in 1–5 ml of hexane is added. After mixing, the hexane is evaporated under suction using continuous agitation on a Whirli mixer to accelerate the process. After the addition of about 0.5 ml of a 5% solution of $LiAlH_4$ in diethyl ether and virtually complete evaporation of the latter solvent, the reaction tube is cooled in liquid nitrogen, sealed off and placed in the reaction

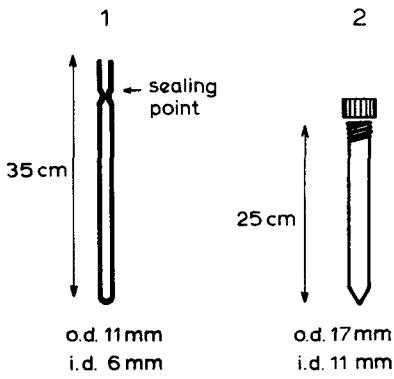

FIGURE 1 Reaction tubes for dechlorination according to (left) Method I, and (right) Method II.

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oven. Here, dechlorination takes place at about 170–180°C, reaction taking 3–4 hr. After cooling of the tube to room temperature and subsequent cooling in liquid nitrogen for 5 min, the tube is opened carefully and one proceeds as described below under Method II.

Method II. 1.0 ml of n-dodecane is added to a glass reaction tube (for dimensions, see Figure 1–2) that can be stoppered with a PTFE-lined screw cap, and mixed with 1–5 ml of a sample extract in hexane. The hexane is completely evaporated in a stream of dry nitrogen; then about 25 mg of dry $LiAlH_4$ and, next, 20–200 μl of anhydrous diethyl ether are added. After rapid mixing, the tube is immediately closed and put into an oil bath in the manner described above. The reaction is performed at a temperature of 170–180°C, either for 3–4 hr or overnight.

After cooling the tube to room temperature, its screw cap is taken off and 5–10 ml of a 20% sulphuric acid solution are added. Care should be taken to add the first milliliters dropwise and under gentle mixing. Next, the biphenyl formed during dechlorination is extracted by adding 5 ml of hexane and shaking for 1–2 min on a Whirli mixer. An aliquot of the organic extract is now transferred to a calibrated centrifuge tube with a pasteur pipette. If the total extract is required for analysis, a second extraction with hexane is performed and the extracts are combined. After evaporation to 1 ml (the amount of dodecane present) and drying over anhydrous sodium sulphate, 100-μl aliquots are injected into the LC system.

In the case of (chlorinated) hydroxybiphenyls, hexane is replaced as extractant by hexane-diethyl ether (1:1), which quantitatively extracts the monohydroxybiphenyls. After evaporation of the extract to 1 ml (dodecane!), the hydroxybiphenyls are re-extracted into aqueous 0.1 M NaOH. The aqueous phase is neutralized and enough methanol is added

to obtain a mixture having a composition roughly equal to that of the mobile phase.

Sample preparation

All details on the preparation and clean-up of the PCB- and PCT-containing samples discussed in the present paper, and on the technique of perchlorination with antimony pentachloride are reported in Ref. 9.

RESULTS AND DISCUSSION

Literature survey

About two decades ago dechlorination, also called hydrodechlorination, of several types of organic compounds over a heated catalyst in a stream of hydrogen gas to obtain the respective parent hydrocarbons was suggested[10] as an analytical technique and developed[11,12] for use in GC. In 1971, Asai *et al.*[13] applied the procedure to the analysis of, amongst other compounds, PCBs. Subsequently, Zimmerli[14,15] used so-called carbon-skeleton GC for the study of PCBs, PCTs, polychlorinated naphthalenes (PCNs) and other classes of compounds. He showed that PCB mixtures are converted quantitatively into biphenyl on a partly deactivated palladium catalyst; under these conditions, polychlorinated terphenyls and naphthalenes are converted into a mixture of the isomeric terphenyls, and into naphthalene plus some tetralin, respectively. Other dechlorination agents were used by various groups of workers[16–18] and the technique was applied for e.g. the structure identification of sample constituents of the technical chloroalkylbiphenyl product Chloralkylene 12 (Ref. 16) and for the analysis of Toxaphene in soil.[17] Further progress in the execution of carbon-skeleton GC was made by Cooke and coworkers.[19–23] They introduced catalytic on-column dechlorination which can be carried out with conventional GC equipment after slightly modifying the apparatus. Packed as well as capillary columns were stated to be compatible with the on-column principle. Successful dechlorinations were carried out with polychlorinated aromatic and aliphatic hydrocarbons. Depending on the type of compound to be dechlorinated, either palladium or platinum was used as catalyst.

Despite such application and innovation, dechlorination has not gained widespread acceptance. This may well be due to the fact that preparation of a suitable catalyst and dechlorination itself do not always appear to be simple procedures. Besides, the extremely high sensitivity, and probably also good selectivity, inherent in, for example, perchlorination no doubt also plays an important role. In 1979, however, to our opinion a rather promising procedure was published by Seidl and Ballschmiter,[24] who

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combined dechlorination with $LiAlH_4$ with column liquid chromatography using UV detection, and claimed the method to be comparable in sensitivity to perchlorination. Their experimental set-up has been used as the starting-point for our investigation. This pursued, as its main object, the application of the technique to a wide range of environmental samples in an attempt to settle the longstanding controversy of pattern comparison versus perchlorination referred to above by elaborating an independent check method.

Dechlorination procedure

Preliminary work was directed at simplifying the dechlorination procedure described by Seidl and Ballschmiter,[24] the aim being to make it experimentally more simple and, thus, a better alternative for, e.g., perchlorination.

To our opinion, the most critical point in dechlorination according to Seidl and Ballschmiter is that the vapour pressure in the reaction tubes—which, next to PCBs, contain dodecane, hexane and diethyl ether—should not be too high. This problem can be partly solved by sucking off the vapours in the headspace of the tube after having it cooled in liquid nitrogen.[24] We successfully tried to omit the evacuation step by (1) evaporating all of the hexane after the dodecane had already been added to the reaction tube (thereby preventing loss of PCBs due to evaporation) and (2) evaporating the major portion of the diethyl ether after addition of the reagent solution. Using this procedure (Method I), good recoveries (see next section) were obtained in the dechlorination of standard PCB mixtures.

Another, rather disagreeable aspect of the original method is the need of having to seal off the reaction tube while cooling it in liquid nitrogen—the tube therefore being of rather excessive length (70 cm). On the basis of the satisfactory results obtained when omitting the evacuation step and, besides, relying on our experience gained in high-temperature perchlorination studies, it was decided to attempt dechlorination in the PTFE-lined screw-capped reaction tubes (Figures 1–2) routinely used in our laboratory for perchlorination.[9] As a further simplification, dry $LiAlH_4$ and diethyl ether (for proper amount, see discussion below) were added separately. Recoveries obtained with the final version of the method (Method II) typically were about 95% (see also below).

Polychlorinated biphenyls

Initially, dechlorination according to Method I was studied with Aroclor 1242 (1–10 μg) as test mixture. At a temperature of 170–180°C, the same as that used by Seidl and Ballschmiter, a reaction time of 2 hr was found

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to yield recoveries of $85 \pm 10\%$, while an increase to 3 hr improved the results to $95 \pm 5\%$. Despite these promising figures, which agree with those reported by the quoted authors, Method I was rejected. Although it is experimentally more simple than the original procedure it is, to our opinion, rather laborious and not too safe in inexperienced hands and, thus, not suitable for routine analysis.

As for Method II, early experiments, again at 170–180°C, were carried out in the reaction oven used with Method I. Recoveries were comparable to those previously obtained—e.g., 85% for Aroclor 1242 after a 1–2 hr reaction—but leaking problems and hardening of the PTFE linings in the screw caps made us change over from the oven to an oil-bath arrangement as discussed in the section on apparatus. As a further modification, instead of using a $LiAlH_4$ solution in diethyl ether, the reagent was now added in dry form and, subsequently, a controlled amount of solvent was added. This change was introduced because in the absence of a sufficient amount of ether dechlorination was found to be rather sluggish. From the data in Table I one can read that the presence

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TABLE I

Dechlorination of PCB (Aroclor) mixtures

Conditions		Recovery (%) of		
Reaction time (hr)	Diethyl ether (ml)	A 1242	A 1254	A 1260
3	0.20	76	73	84
15	0.20	92	91	101
15	0.05	78	77	75

of about 200 μl of ether results in 90–100% recovery (also observed for biphenyl itself) for an overnight reaction which should be preferred to a 3 hr one. For the rest we note that the extraction of biphenyl—after the decomposition of the excess of $LiAlH_4$ with a sulphuric acid solution—can conveniently be carried out in the reaction tube; i.e., a transfer step and, thus, possible loss of biphenyl, is avoided.

Adsorption LC is carried out in the silica gel/dry hexane system extensively discussed by Brinkman *et al.*[25,26] The UV detection limit for biphenyl at 248 nm ($k' = 10$–11; $\log \varepsilon_{248} = 4.3$) is about 0.5 ng. Detection at 205 nm admittedly gives an about 2-fold increase in signal intensity ($\log \varepsilon_{205} = 4.6$), but the baseline noise also shows a distinct increase, the net result being that the signal-to-noise ratio at both wavelengths is

approximately the same (cf. Figure 2). Since in real samples more interferences will generally show up at 205 nm, 248 nm was preferred as wavelength of detection. The detection limit of biphenyl in LC-UV (i.e., with dechlorination) is about 3 orders of magnitude higher than that of decachlorobiphenyl in GC-ECD (i.e., with perchlorination). One should realize, however, that the injection volumes in LC and GC typically are about 100 and 1–5 μl, respectively. This implies that, when replacing perchlorination by dechlorination, the actual loss in sensitivity is slightly over one order of magnitude. Dechlorination is, on the other hand, more sensitive than is the GC-ECD pattern-comparison method.

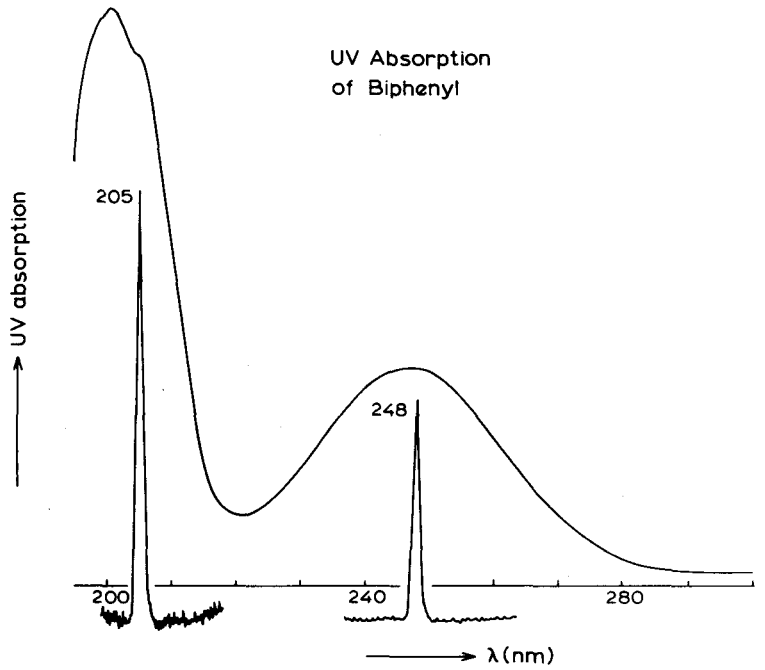

FIGURE 2 UV-absorption spectrum and LC-UV detection of biphenyl at 205 and 248 nm.

Dechlorination as a method for quantitative analysis of PCBs in various types of samples was evaluated and compared with results obtained via pattern comparison and perchlorination, which often yield remarkably different results. Data for a series of recycled paper, toilet paper, heron liver and fat, and fish (sprat and eel) samples are presented in Table II. In the case of the paper samples, the PCB pattern invariably is closely analogous to that of Aroclor 1242, while with the biological samples Aroclor 1254/1260 or Aroclor 1242/1254/1260 mixtures had to be

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TABLE II

Comparison of PCB levels in environmental samples as determined by pattern comparison (PC), perchlorination (PE) and dechlorination (DE)[a]

Sample		Pattern comparison (μg PCB/g)			Perchlorination (μg DCB/g)	Dechlorination (μg biphenyl/g)	Ratio	
		A1242	A1254	A1260			PE/PC	DE/PC
Toilet paper	I	0.16			0.59	0.07	2.0	0.8
	II	0.32			6.0	0.20	9.8	1.0
	III	1.90			11.0	0.93	3.0	0.8
	IV	5.28			49.3	4.50	4.8	1.4
Recycled paper	I	0.53			3.4	0.27	3.4	0.9
	II	0.55			12.0	0.25	10.9	0.8
	III	0.77			37.5	0.55	25.5	1.2
Heron fat	I		11.5	11.5	65.0	10.1	1.9	0.8
	II		126.0	126.0	361.0	121.0	1.0	1.1
	III		10.2	10.2	47.0	9.0	1.6	1.0
liver	IV		0.7	0.7	8.2	0.6	3.9	1.0
Fish[b]	sprat	6.1	19.5	13.2	60.0	22.0	1.0	1.2
	eel	1.7	5.0	4.6	15.0	5.0	0.9	1.0

[a]Conversion factors for Aroclor 1242, 1254 and 1260 if going from pattern comparison to dechlorination are 0.59, 0.47 and 0.41, respectively; if going from pattern comparison to perchlorination, the values are 1.91, 1.54 and 1.35, respectively.
[b]Calculated on fat weight basis.

used as standards. It is evident that, with the paper samples, dechlorination and pattern comparison yield closely analogous results—with mutual differences which can be expected within the range of variation of environmental samples—and that the perchlorination data are from 2- to 25-fold higher. In the case of the biological samples, the agreement between perchlorination and pattern-comparison data is more satisfactory, but exceptions do occur. No discrepancies are, however, noted in the case of dechlorination versus pattern comparison. Here, one should realize that with the biological samples the PCB levels are relatively high; this will easily obscure any discrepancies still present between perchlorination and pattern comparison. In this regard, it may be important that the single major exception shows up with the sample (heron liver) having a rather low PCB concentration level.

Typical examples of chromatograms of extracts obtained after dechlorination are shown in Figure 3 for a toilet paper and a heron fat sample. The latter one gives a remarkably "clean" chromatogram, which no doubt is at least partly due to the high PCB level. With the paper

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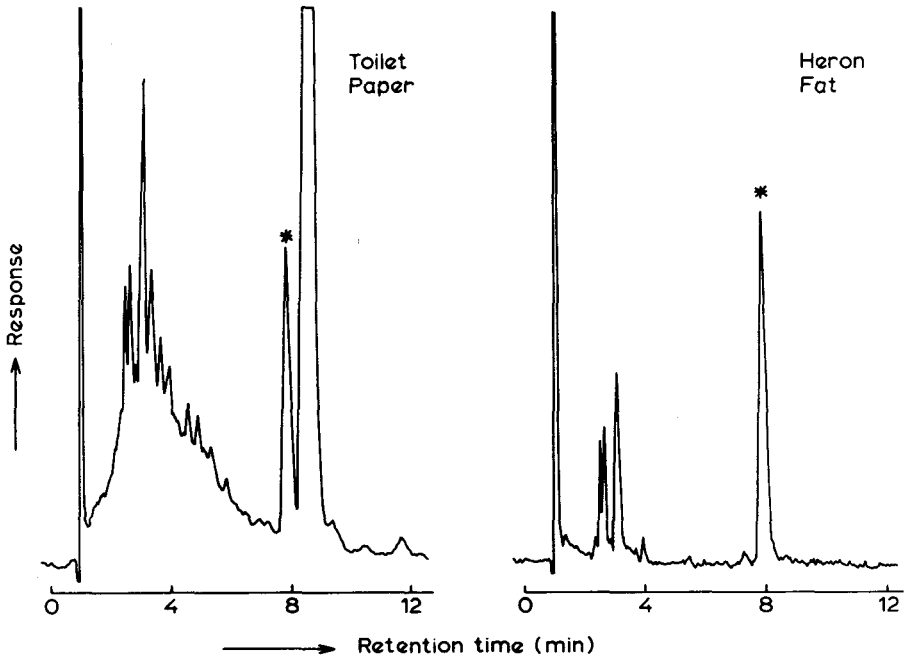

FIGURE 3 LC-UV (248 nm) chromatograms of dechlorinated PCB-containing extracts of a toilet paper and a heron fat sample. Asterisk denotes the biphenyl peak.

sample, a large peak shows up which has a slightly higher capacity ratio than has biphenyl. The sample constituents causing the occurrence of this peak have, meanwhile, been shown to be partly hydrogenated terphenyls; these appear to be mainly responsible for the erroneous results often found with perchlorination.[9]

It is self-evidently advisable to record LC chromatograms both before and after dechlorination in order to prove the absence of biphenyl from the sample itself. Chromatograms for a rather complex toilet paper mixture are presented in Figure 4. According to expectations the peaks due to the partly hydrogenated terphenyls again show up, both prior to and after dechlorination.

It is evident that the validity of the dechlorination procedure will have to be checked for each new type of sample. Should separation problems

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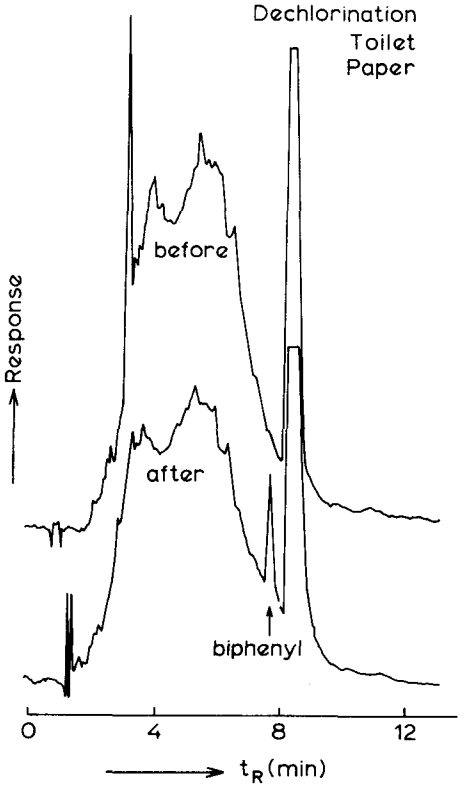

FIGURE 4 LC-UV (248 nm) chromatograms of a PCB-containing toilet paper sample extract before and after dechlorination.

arise, then an additional clean-up will be necessary. For example, in the system silica gel/dry hexane any biphenyl originally present in the sample can conveniently be separated from all individual PCBs (cf. Ref. 25). Alternatively, reversed-phase LC in the system LiChrosorb RP-18/methanol-water can be used, in which case biphenyl elutes prior to, instead of after, many chlorinated aromatics (inclusive PCBs).[27] As for interfering compounds, PCTs do not form any appreciable amount of biphenyl on dechlorination (cf. next section). The same holds true for PCNs: the commercial mixture Halowax 1014 was found to be converted into naphthalene. Since naphthalene is much less retained ($k' = 4.5$) in the silica gel/hexane system than is biphenyl, dechlorination appears to be an elegant means to discriminate between PCNs and PCBs. $LiAlH_4$ treatment of polybrominated biphenyls (PBBs) effects complete debromination, as was observed for the commercial mixture fireMaster BP-6 (mainly consisting of hexabromobiphenyls) and decabromobiphenyl. Partial conversion into biphenyl occurs in the case of chlorinated hydroxybiphenyls, as will be discussed in more detail below. It can well be argued, that in a large majority of all environmental samples the level of PBBs and hydroxylated PCBs will be much lower than that of PCBs, so that serious errors are not to be expected.

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Polychlorinated terphenyls

Dechlorination (to a mixture of o-, m- and p-terphenyl) may well be a reliable method of analysis for PCTs, since quantitation by means of GC is rather cumbersome on account of the low volatility of PCTs and the large number of individual constituents present in the commercial mixtures.

Our studies revealed that virtually complete (over 90%) dechlorination of Aroclor 5432, 5442 and 5460 cannot readily be achieved. When varying the same parameters as in the work on PCBs, recoveries were found to lie between 35 and 70% (Table III). Best results were again obtained with 200, instead of 50, μl of diethyl ether; the effect of temperature—which appears to be contrary to that observed for PCBs—is rather minor if a sufficient amount of ether is present. Increasing (to 200°C) or decreasing (to 140°C) the temperature did not improve the results. The relatively low terphenyl recovery is not easily explained. Rather surprisingly, GC-ECD chromatograms of extracts of dechlorinated PCT samples (50% terphenyl recovery) did not reveal the presence of significant amounts of non-converted individual PCTs. Possibly, partial decomposition of the PCTs occurs upon dechlorination. This hypothesis is confirmed by the observation that overnight "dechlorination" of the three parent terphenyls leads to 10–20% losses. On the other hand, however, no decomposition

TABLE III
Dechlorination of PCT (Aroclor) mixtures

Conditions		Recovery (%) of		
Reaction time (hr)	Diethyl ether (ml)	A 5442	A 5422	A 5460
3	0.05	55	50	45
15	0.05	45	—	35
3	0.20	65	55	70
15	0.20	70	60	50

was ever observed with the PCBs; besides, it was found that only a negligible amount of biphenyl is formed upon dechlorination of PCTs.

Since the studies on PCBs and PCTs were carried out simultaneously, the same chromatographic system was used for analysis. This implies that the capacity ratios of the parent terphenyls are fairly high (ortho, 28; meta, 20.5; para, 21). Therefore, a high flow-rate of about 2.5 ml/min was used for PCT analysis. PCB analyses were done at around 1.5 ml/min, since at the higher flow-rate incomplete separation of the biphenyl peak from neighbouring peaks was occasionally observed. For the rest one should realize that with PCTs the time of analysis can be reduced by the addition of a small amount of modifier (water) to the mobile phase. Under such conditions, however, the separation between the three terphenyls is (partly) lost.

Detection of the terphenyls can conveniently be done by means of UV-absorption measurements. Next to a fairly strong absorption band at around 210 nm, the isomers display a characteristic band at 232 (ortho), 245 (meta) and 274 (para) nm, respectively (Figure 5). Selective detection is therefore easily accomplished. The detection limits are, at 2–5 ng, slightly high, which is at least partly due to the unfavourably high capacity ratios quoted above. Besides, conversion into three, instead of one peak(s) makes dechlorination of PCTs somewhat less attractive than that of PCBs.

As an example, the determination of PCTs in a toilet paper sample is shown in Figure 6. Detection at 215 nm, where the response of all three terphenyls is about the same, reveals a very complex chromatogram, in which the three peaks of interest can hardly be recognized. Detection of the peak due to the ortho isomer—which generally is the least abundantly available isomer—is especially difficult. This is also true when detection is performed at 235 nm, another favourable wavelength for the detection of o-terphenyl. The meta isomer, on the other hand, clearly shows up at the latter wavelength, while the para peak is readily visible in the relatively

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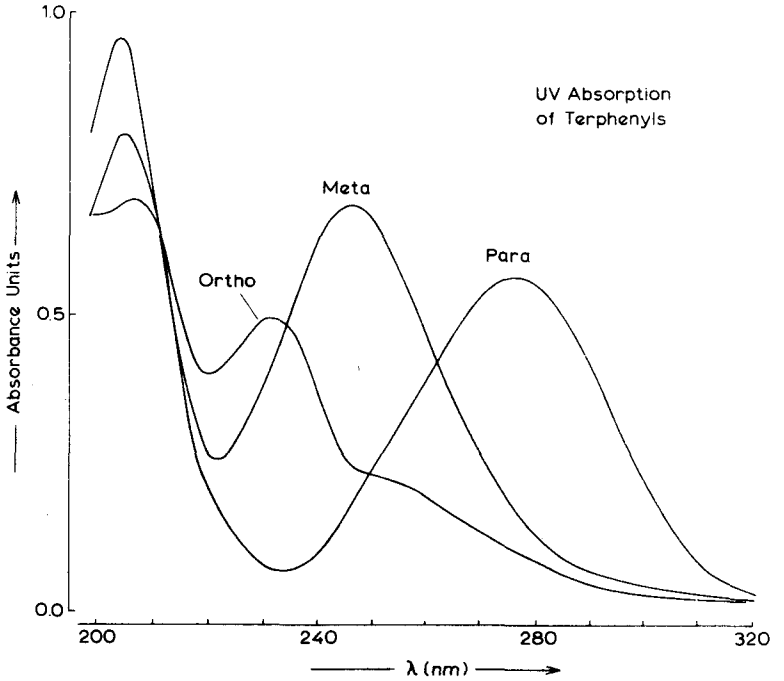

FIGURE 5 UV-absorption spectra of o-, m- and p-terphenyl.

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clean chromatogram recorded at 275 nm. For this particular sample, quantitation was done by comparing the peak heights of the meta and para isomers in the dechlorinated sample with those of a dechlorinated Aroclor 5460 standard (since pattern comparison showed the PCT sample pattern to match that of Aroclor 5460). The PCT level was calculated to be 2.6 μg/g, which is in good agreement with the value of 2.4 μg/g obtained via pattern comparison. Perchlorination, on the other hand, yielded a nearly 3-fold higher result.

Hydroxylated PCBs

A preliminary study was made on the application of dechlorination to the analysis of chlorohydroxybiphenyls, which are well known metabolites of PCBs.

Recovery data for four monohydroxy-PCBs are shown in Table IV. These are average values which have rather large relative standard deviations of about 30%. Conversion to the monohydroxybiphenyls is seen to be between 27 and 42%, with rather minor differences between a

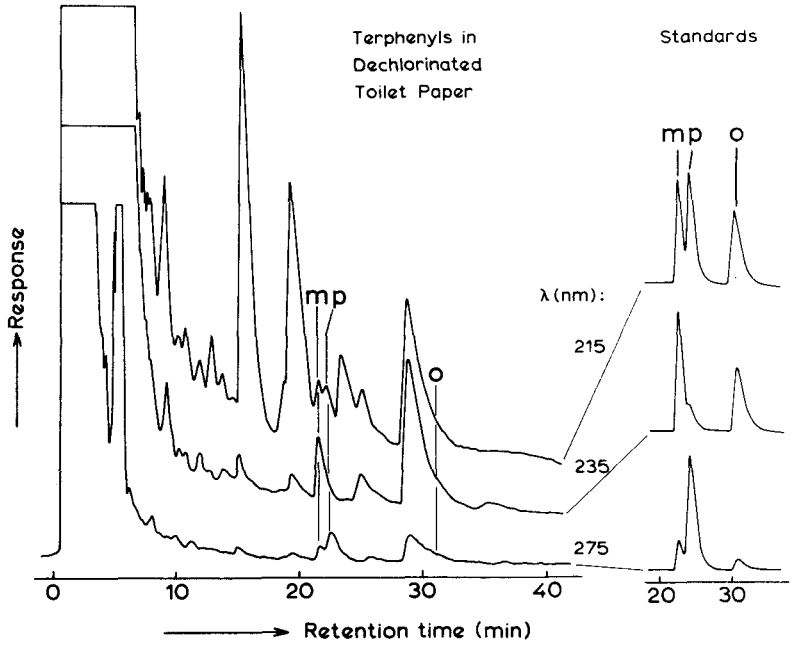

FIGURE 6 LC-UV chromatograms of a dechlorinated PCT-containing extract of a toilet paper sample and of equal weights of o-, m- and p-terphenyl standards. UV detection was done at the indicated wavelengths.

TABLE IV
Dechlorination of (chloro)hydroxybiphenyls

	Recovery (%) of					
	HO-biphenyl		Biphenyl		Orig. compound	
Compound	3 hr	15 hr	3 hr	15 hr	3 hr	15 hr
4'-Cl-4-OH	30	28	38	42	14	4
2-Cl-4-OH	42	27	32	53	8	2
3-Cl-4-OH	38	30	32	39	7	3
5-Cl-2-OH	28	30	47	72	9	2
2-OH	[a]	[a]	29	31	43	38
3-OH	[a]	[a]	31	30	41	40
4-OH	[a]	[a]	37	36	43	44

[a]In the case of non-chlorinated hydroxybiphenyls, the "hydroxybiphenyl" and "original compound" self-evidently are identical.

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3 hr and an overnight reaction. In the latter case the amount of starting product still present is only 2–4% as against 7–14% after 3 hr; however, the significant amount of biphenyl formed has increased. The rather ready conversion into biphenyl is also observed with the monohydroxybiphenyls themselves, as is evident from the data included in the table. Similar results were found with four higher substituted, i.e., monohydroxy(di →penta)chlorobiphenyls: recoveries of the parent monohydroxybiphenyls always were in the range 25–40%.

Obviously, the present dechlorination technique is not suitable for the quantitative analysis of hydroxylated PCBs. The method can, on the other hand, be valuable in qualitative studies of PCB metabolism, dechlorination of an unknown PCB metabolite giving information on the position of the hydroxy group on the biphenyl ring. This is especially interesting as many of the hydroxybiphenyls to be used as standards are readily available, while the large majority of the hydroxylated PCBs is either very expensive or not available at all. The principle is demonstrated in Figure 7. The peaks due to the two hydroxylated PCBs virtually disappear on $LiAlH_4$ treatment and a large peak due to 4-hydroxybiphenyl which is formed upon dechlorination, shows up.

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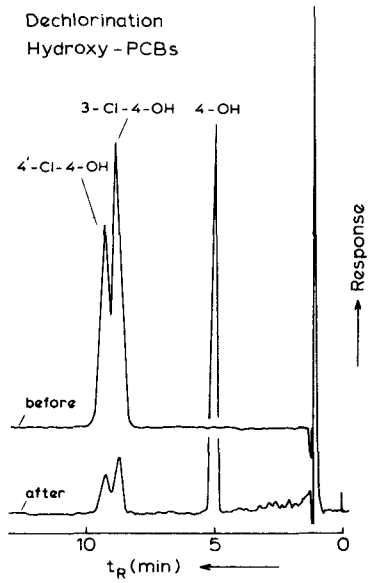

FIGURE 7 LC-UV (265 mm) chromatograms of a mixture of 3-chloro-4-hydroxy- and 4'-chloro-4-hydroxybiphenyl before and after dechlorination. System: LiChrosorb RP-8/methanol-water (7:3).

The above method, which is experimentally more simple than dechlorination/GC procedures reported in the literature,[28,29] has recently been applied by us during a study on the metabolism of 4,4′-dichlorobiphenyl in the rat. 0–24 hr rat urine was collected, acidified with sulphuric acid, and the mixture extracted with hexane-diethyl ether (1:1). The extract was evaporated, redissolved in methanol-water (1:1) and an aliquot injected into the LC system. The major peak showing up in the chromatogram was collected, and, after suitable pre-treatment, subjected to dechlorination. Subsequent analysis according to the above procedure showed the end-product of dechlorination to be 3-hydroxybiphenyl. This provided—in this case, additional—evidence that 4,4′-dichloro-3-hydroxybiphenyl is the main metabolite of 4,4′-dichlorobiphenyl.

CONCLUSION

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Up till now, dechlorination has generally been regarded as a rather complicated and capricious technique. In the present paper, a modified and simplified version of a previously published[24] dechlorination procedure has been elaborated. This makes dechlorination as easy to perform as, for example, perchlorination and thus makes it suitable for routine application in the analytical laboratory. Dechlorination combined with subsequent analysis by means of LC-UV has been shown to be an attractive technique to determine PCB levels in many types of environmental samples. The results are closely analogous to those obtained with GC pattern comparison and, thus, help to prove that PCB levels determined by means of perchlorination indeed often are incorrect.

The present work also demonstrates that dechlorination can be used in the analysis of PCTs—even though the detection limits are less favourable in this case—and in qualitative studies on chlorohydroxybiphenyls, the main application here being structure elucidation.

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